**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| APPLICANTS: | David E. Hill, et al. |
| DOCKET NO.: | 1266.0 |
| SERIAL NO.: | 10/689,316 |
| CONFIRMATION NO.: | 4126 |
| FILED: | October 20, 2003 |
| ART UNIT: | 1743 |
| EXAMINER: | Hyun, Paul Sang Hwa |
| TITLE: | Enhanced Monitor System for Water Protection |

# DECLARATION under 37 C.F.R. 1.131 of Prior Invention in the United States To Overcome Cited Patent or Publication

Commissioner for Patents
Mail Stop Non-Fee Amendment
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

## PURPOSE OF DECLARATION

This declaration is to establish completion of the invention in this application in the United States, at a date prior to July 24, 2003, that is the effective date of the McCarter U.S. Patent Application Publication 2005/0065755 that was cited by the examiner.

**FACTS AND DOCUMENTARY EVIDENCE**

To establish the date of completion of the invention of this application, the following attached document is submitted as evidence:

UT-Battelle *Report of Invention* submitted by the applicants and received in electronic form, printed, and signed as received by Joseph A. Marasco, UT-Battelle Registered Patent Agent, on May 16, 2003.

From this document, it can be seen that: The invention claimed in the above identified patent application was conceived and reduced to practice at least by the date of May 16, 2003, which is a date earlier than the effective date (July 24, 2003) of the reference.

**DILIGENCE**

On May 16, 2003, the applicants diligently submitted a complete *Report of Invention (ORNL-40)* to Joseph A. Marasco, Registered Patent Agent for UT-Battelle, in accordance with company procedure, thereby establishing a reported date for complete conception and actual reduction to practice of the invention. The *Report of Invention* contains references to inventor's notebooks that may establish earlier dates of conception and actual reduction to practice, but the earlier dates are not needed for this declaration.

Actual reduction to practice is further supported by constructive reduction to practice.

Until May 20, 2003, Joseph A. Marasco diligently proceeded to collect pertinent facts and information about the invention, at which time, UT-Battelle disclosed the invention to Emily G. Schneider, Assistant Chief Counsel for Intellectual Property at the Department of Energy Oak Ridge Operations Office, in accordance with DOE contract terms.

Until July 21, 2003, UT-Battelle diligently pursued election of invention rights, at which time DOE granted election to UT-Battelle for retention of rights and commercialization.

Until October 20, 2003, Joseph A. Marasco diligently prepared the patent application, at which time the application was filed thereby establishing constructive reduction to practice.

**TIME OF PRESENTATION OF THE DECLARATION**

This declaration is submitted prior to final rejection.

**DECLARATION**

As a person signing below:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

/Joseph A. Marasco/

Joseph A. Marasco
Patent Agent of Record
Reg. No. 32,798
UT-Battelle, LLC
P.O. Box 2008
Oak Ridge, TN 37831-6258
865-574-4178

**OAK RIDGE NATIONAL LABORAT   Y**
MANAGED BY UT-BATTELLE, LLC
FOR THE U.S. DEPARTMENT OF ENERGY
Under Contract No DE-AC05-00OR22725

# REPORT OF INVENTION

ORNL-40 *Electronically fillable in MS WORD* (October, 2002)

**Intell   ual Property Section Use Only**

Disclosure No. 1266

DOE S- 101, 849

*INSTRUCTIONS: Place pointer over highlighted text to reveal instructions here and throughout this document.*

## PART I - DESCRIPTION OF THE INVENTION

1. SUBMITTER(s). David E. Hill, Miguel Rodriguez, Jr., Elias Greenbaum, and James W. Klett

2. TITLE:
AquaSentinel$^{SM}$ Buoy

3. BRIEF DESCRIPTION OF THE INVENTION:
AquaSentinel$^{SM}$ Buoy is a remotely controlled, buoyant device for detecting toxic agents in sunlight-exposed waters using chlorophyll fluorescence monitoring. This invention overcomes the problem of remote rapid detection of toxic agents in water under real-world conditions. Recent terrorist attacks in the United States have increased the awareness of the need for ways to protect drinking water supplies. Source waters for civilian populations and military facilities are vulnerable to such attacks. This invention is designed to make rapid remote assessments of possible contamination of source waters by toxins prior to entry to drinking water distribution systems.

4. BACKGROUND:
Field of the invention

AquaSentinel$^{SM}$ Buoy is a revolutionary approach to detecting toxic chemicals in large-scale primary-source drinking water supplies (reservoirs, rivers, lakes, etc.). It provides around-the-clock unattended monitoring and uses naturally occurring aquatic photosynthetic tissue as the sensing material. This biosensor technology can be used as a first-alert warning system for toxic agents' attacks on municipal and military drinking water supplies.

Using fundamental principles of photosynthesis combined with state-of-the-art optoelectronic instrumentation, the AquaSentinel$^{SM}$ Buoy can provide 24-hour/7-day unattended protection of all sunlight-exposed primary-source drinking water supplies.
The new system is considered a complementary technology to the standard battery of water quality laboratory analyses of municipal drinking water, and it is not meant to replace this service. Rather, it is a broad-spectrum continuous monitoring instrument that is designed to provide an early warning alert signaling that municipal or military drinking water supplies have been exposed to potentially harmful chemical agents. The most significant aspect of this biosensor technology is the use of the algae and phytoplankton populations that occur naturally in all sunlight-exposed drinking water as the biosensor material (Rodriguez, et al., 2002; Greenbaum and Sanders, U.S. Patent Application, Publication Number US 2002/0102629). Using these naturally occurring biosensors provides considerable practical and cost benefits for implementation of this technology in a real-world setting (Sanders, et al., 2001; Greenbaum and Sanders, U.S. Patent Application, Docket 0815.~~X~~).

Description of the Prior Art

To our knowledge, there is no commercially available field-deployable system that has the robustness, secure wireless data transmission, and mobility of the AquaSentinel$^{SM}$ Buoy. The closest competitive products are the Algae Toximeter® sold by bbe Moldaenke GmbH, Kiel-Kronshagen, Germany (Moldaenke and Beutler, 2001; European Patent #DE19941415 and Moldaene, 1993; European Patents #WO09312415 and #EP0615618) and the Fluotox® water pollution biosensor sold by Arnatronic, Ltd., Arnaville, France (Ory, 1997). Both systems are used for standard water quality monitoring. These products are static non-submersible bench-top instruments that use laboratory-cultured algae as the biosensor material. The use of cultured algae requires a great deal of labor-intensive maintenance, and these cultured biosensors have a short time-span of usability. In sharp contrast, the AquaSentinel$^{SM}$ Buoy is a robust and mobile field-deployable instrument that uses naturally occurring algae as the biosensors. Information is transferred by encrypted wireless telecommunications to state-of-the-art analytical software and database libraries for quick analysis.

AquaSentinel℠ Buoy is a tail-tube buoy. Tail-tube buoys consist of a vertical tube surrounded by a concentric, cylindrical flotation collar. The relative oscillation between the water column inside the tube and the buoy hull can be used to absorb energy from the wave field surrounding the buoy (Korde, 2000).

Kishino, et al. (1997) used data from a moored optical buoy system to verify satellite data. Spectral incident irradiance was measured at top of the buoy system. Phytoplankton pigment concentrations were monitored by Aquatracka-III fluorometer placed at the bottom of the surface float. Atmospheric temperature, pressure, humidity, wind speed and direction, water temperature and wave height and direction were also monitored.

Wild-Allen, et al. (1997) used drifting buoys with 4-channel downward-looking color sensor with chlorophyll concentration for case I waters near the Canary Islands. Chlorophyll fluorescence profiles were measured with a Seatech 102S fluorometer at the start and end of each buoy deployment, and water samples were taken with a Rosette water sampling system at several depths.

Dubelaar, et al. (1999) reported the development of a small, wireless battery-operated in situ flow cytometer for autonomous operation inside a small moored buoy. Since it was designed to operate in a buoy, they called it CytoBuoy. CytoBuoy allowed on-line in situ particle analysis, estimation of phytoplankton biomass, and discrimination between different phytoplankton groups. Sampling, analysis, and radio transmission of the data proceeded automatically at user-defined intervals. A powerful feature was the acquisition and radio transmission of full detector pulse shapes of each particle.

Moore, et al. (2000) developed a system for continuous monitoring of river level using a buoy equipped with Global Positioning System (GPS) and satellite communications and using Geographical Information System (GIS) techniques. They conducted field trials using a buoy simulator and a prototype integrated buoy system. The system consisted of the following components: 1) A river buoy with a GPS receiver, pressure and tilt sensors, and an autonomous power supply, 2) Two-way data link (terrestrial) to a nearby GPS reference station, 3) A GPS reference station with satellite or terrestrial communications capability, and 4) A central station with the hydrological database and the integrated GIS system. From their field trials results, they concluded that GPS offered the capability of monitoring river heights in real time.

Pinto, et al. (2001) reported continuous measurement of chlorophyll-a concentration due to plankton, in surface water from two locations in Brazil. The measuring system consisted of a Turner 10-AU fluorometer equipped with a blue lamp, an excitation path filter, and a 680 nm emission filter. For their field studies, they placed the fluorometer and data logging and positioning equipment aboard a light boat that was steered by GPS. The fluorometer was operated in a continuous flow mode with the water flowing through a 25 mm cell. The system was driven by the battery of the boat. Two opaque hoses were attached to the extremities of the cell. One of them was connected to a submersed feed pump whereas the other discharged the outflow, and occasionally was used for collecting samples for spectrophotometric laboratory measurements needed for calibration purposes. The pumping flow rate was fixed at 32 l/min, and fluorometer readings were logged at 5 s intervals. At the end of each run, the whole set of data stored in the fluorometer's memory were transferred to a notebook computer for later processing.

Gelda and Effler (2002) used robotic buoys in Onondaga Lake, NY. The monitoring program included measurements of dissolved oxygen (DO), temperature (T), and fluorometric chlorophyll (Chl) a in the lake's surface waters. The computer-driven robotic monitoring buoys were described as RUSS units (Apprise Technologies®). The underwater probe package, or sonde, that contained the sensors, was exchanged weekly with a newly calibrated and maintained sonde. Robotic measurements were checked weekly with manual measurements to very performance. An on-board computer controlled operations of the buoy including data collection and storage, profiling strategies (e.g., frequency, depth), and communications with the base station (on shore) computer. Commands for measurements were sent from the base station to the buoy via cellular phone. The buoys were solar powered.

Lapota, et al. (2002) described the use of an autonomous bioluminescence buoy (Biobuoy) in the San Diego Bay area. The buoy was composed of three parts: float, oil spill sensor and bioluminescence-water clarity sensor. The oil spill sensor had a fluorescence-based sensor that operates from just below the water's surface and was continuously measuring for an increased concentration of hydrocarbons or a surface sheen, which is indicative of an oil spill. The greater the fluorescence, the higher the concentration of hydrocarbons present. The sensor was able to discriminate between various classes of oils and fuels.

The bioluminescence sensor system consisted of a bioluminescence sensor and a transmissometer. Bioluminescence was measured by a Hamamatsu photomultiplier tube viewing a light-tight darkened 25-mL volume chamber. Seawater was continuously pulled into the chamber by an electric pump at a constant rate of 0.25L/sec. The turbulence associated with seawater mixing in the chamber stimulated the bioluminescent plankton (single-cell dinoflagellates) to emit light. A SeaTech red transmissometer (680 nm) was also mounted in the BioBuoy and provided water clarity or percent transmission (%T) measurements. Data was automatically collected twice an hour, transmitted by a spread spectrum (900 Hz) RF link to a lab-based computer, and posted to a secure internet browser.

Data was logged to a time series graph a... posted to a data file for offloading. Position, ti..., bioluminescence (photons/sec/mL of seawater), %T, and temperature in °C (where available) were logged at every update.

Grana and Haynes (U.S. Patent #4,089,209) described a remote water monitoring system that integrates the functions of sampling, sample preservation, sample analysis, data transmission and remote operation. The system employs a floating buoy carrying an antenna connected by lines to one or more sampling units containing several sample chambers. Receipt of a command signal actuates a solenoid to open an intake valve outward from the sampling unit and communicates the water sample to an identifiable sample chamber. Such response to each signal receipt is repeated until all sample chambers are filled in a sample unit. Each sample taken is analyzed by an electrochemical sensor for a specific property and the data obtained is transmitted to a remote sending and receiving station. Thereafter, the samples remain isolated in the sample chambers until the sampling unit is recovered and the samples removed for further laboratory analysis.

Sutherland and Swenson (U.S. Patent #4,448,068) reported the invention of a shallow water environmental/oceanographic measurement system for real time monitoring of specific parameters at a particular location. A three point moored buoy is anchored over the particular location. Bottom mounted sensors to measure the specific parameters are situated generally within the area of the three point mooring area. Sensor data is transmitted via cable to the buoy from whence it is telemetered to a shore station.

Baxter (U.S. Patent #5,532,679) claimed the invention of an oil spill detection system to detect and track oil spills and transmit data to a remote monitoring center comprising a buoyant detection platform including at least one detection sensor to detect the presence of hydrocarbons in the water adjacent the buoyant detection platform and generate a detection signal in response thereto and a communication system operatively coupled to the detection sensor to receive the detection signal and to generate a data signal in response to the detection signal and to transmit the data signal to the remote monitoring center, the data signal including a discrete platform identification signal corresponding to the buoyant detection platform, and a deployable free floating tracking buoy to be deployed when the detection signal is generated to indicate the direction and speed of travel of the oil slick created by the oil spill.

Baxter and Bailey (U.S. Patent #5,654,692) described a tracking buoy including a sensor to detect the presence of hydrocarbons in a body of water and a communication system to transmit data to a remote monitoring center when the hydrocarbons are detected to indicate the direction and speed of travel of the hydrocarbons in the body of water. The tracking buoy comprises a lower housing and an upper cover to operatively support an antenna, a tracking sensor means and a tracking buoy mounting means to operatively house a power source, a tracking buoy control and a tracking buoy transmitter. The tracking buoy further includes a tracking buoy retainer/release mechanism operatively mounted to a platform by an attachment member to selectively mount and release the tracking buoy to and from the platform.

The tracking sensor means comprises at least one tracking sensor comprising a state of the art fluorometer including an automatic temperature composition means capable of detecting and measuring hydrocarbons, petroleum and petroleum by-products in the parts-per-billion range when present in the water adjacent the tracking buoy. An oil spill detection system may comprise a stationary platform anchored in the water for use with a remote control center and the tracking buoy to be deployed when an oil slick is detected. The stationary platform includes a sensing means to generate a detection signal when hydrocarbons are sensed to release the tracking buoy including a radar reflector or a transmitter/receiver combination and a communication system to generate a data signal including date, time and site location for transmission to the remote control center when the hydrocarbons are sensed.

Lobsiger and Manuel (U.S. Patent #6,119,630) reported an installation for in situ monitoring the quality of a habitat of aquatic organisms with minimal disturbance to the aquatic organisms. In a first aspect of the present invention, the monitoring installation uses invertebrate sentinel species such as mussels, clams, oysters and scallops. The monitoring installation comprises broadly, a buoy, a mooring and a monitoring apparatus comprising; a framework having an upper end connected to the buoy and a lower end connected to the mooring. There is also provided a camera attached to the framework. The camera is adapted for underwater operation and has image data generating and transmitting capabilities for transmitting a number of images to a remote receiver. A support structure is detachably mounted to the framework and has rotary retainers for movably supporting one or more socks of mollusks in front of the camera. This invention relates to early warning systems for monitoring coastal water quality, and more particularly, it relates to a portable submersible apparatus for in situ monitoring the quality of habitat of aquatic organisms, or for in situ detecting lethal and sublethal pollution in inland or sea water.

5. DETAILED DESCRIPTION OF THE INVENTION:

AquaSentinel[SM] Buoy is a field-deployable buoyant monitoring device for around-the-clock monitoring of drinking water, and an early warning alert for a terrorist act, or an accidental toxic spill. The buoy is a self contained laboratory providing continuous updates to a central command center for data processing. Any non-porous material can be used for the buoy's housing, but the preferred embodiment is the graphite fiber composite (Klett and Edie, 1995; Klett, et al., 2000). This disclosure includes designs of two buoy models: 1) light sediment model and 2) heavy sediment model.

*Light Sediment Model* (see Figure 1)

Components:

1: Wireless antenna
2: Control circuitry
3: Thermally insulated covering
4: Stabilizing wing
5: Waterproof seal
6: Intake tube
7: Thermally conductive covering
8: Pump (with check valve)
9: Power supply (battery storage)
10: Exhaust output tube
11: Fluorometer with sample cuvette
12: Solar panel

Detailed operation:

1. Power source

Power supplying the buoy is a solar panel (12) that charges a deep cycle battery located in the power supply (9). The control circuits (2) continually monitor the power level to detect low battery and failure conditions.

2. The AquaSentinel℠ Buoy fluid cycle.

When the pump (8) is activated, the water enters in through the intake tube (6) to allow a fresh water sample the fluorometer's (11) cuvette.

The pump (8) will continue running long enough to allow the water to be completely replaced in the tubing, purging any sediment that may have built up during the previous cycle. The pump (8) stays off long enough for the dark adaptation of the algae and to allow the clarification of sediment. The total time the algae are in the dark is approximately 4 minutes.

3. Detection:

The xenon lamp will flash and detectors measure the variable fluorescence every 3-4 minutes. The data can be sent to a central control center via the wireless antenna

4. Other key features:
   - Thermally conductive lining reduces the risk of overheating/freezing of the water in and around the buoy.
   - If temp <0c pump runs continually
   - Heat sink on the pump connected to intake pipe to prevent freezing (Winter) and cool system (Summer)
   - Sends data to wireless network port

*Heavy Sediment Model* (see Figure 2)

Components:

1: Wireless antenna
2: Control circuitry
3: Thermally insulated covering
4: Stabilizing wing
5: Waterproof seal
6: Thermally conductive covering
7: Small reservoir (50ml staging area 2 preferably)
8: Fluorometer with sample cuvette
9: Intake tube
10: Power supply (battery storage)

11: Pump
12: Stage selector valve (three-way)
13: Large reservoir (200ml staging area 1 preferably)
14: Exhaust output tube
15: Air purge valve (normally closed)
16: Air purge tube
17: Solar panel

Detailed operation:

1. Power source

Power supplying the buoy is a solar panel (17) that charges a deep cycle battery located in the power supply (10). The control circuits (2) continually monitor the power level to detect low battery and failure conditions.

2. The AquaSentinel[SM] Buoy fluid cycle.

The water enters in through the intake tube (9) and enters the large reservoir (13). This staging area will fill by a siphoning effect as long as the air purge (15) valve remains open. After the initial filling of the buoy this valve (15) remains closed except for an occasional air purge.

The secondary staging area (7) will fill only if pump (11) is running and the stage selector valve (12) is engaged allowing flow from the fluorometer (8). The stage selector valve (12) is a three way valve that is engaged when it is open to the fluorometer and disengaged allowing flow from the large reservoir (13).

At the beginning of the cycle, the pump (11) turns on. The stage selector valve (12) opens to allow a fresh water sample into the small reservoir (7) and the fluorometer's (8) cuvette. The stage selector valve (12) then switches back to move water through the large reservoir (13) and the pump will continue running long enough to allow the water to be completely replaced in the large reservoir (13), purging any sediment that may have built up during the previous cycle. The pump(11) stays off long enough for the dark adaptation of the algae and to allow the clarification of sediment . Since the water is replaced in the small reservoir (7) at the beginning of the cycle, the total time the algae are in the dark is approximately 4 minutes.

3. Detection:

The xenon lamp will flash and detectors measures the variable fluorescence every 3 minutes or so. The data can be sent to a central control center via the wireless antenna

4. Other key features:
   - The Large reservoir (13) reduces the amount of normal sediment from collecting in the Fluorometer's Cuvette.
   - Thermally conductive lining reduces the risk of overheating/freezing of the water in and around the buoy.
   - If temp <0c pump runs continually
   - Heat sinks on the pump and valves to intake pipe to prevent freezing (Winter) and cool system (Summer)
   - Sends data to wireless network port

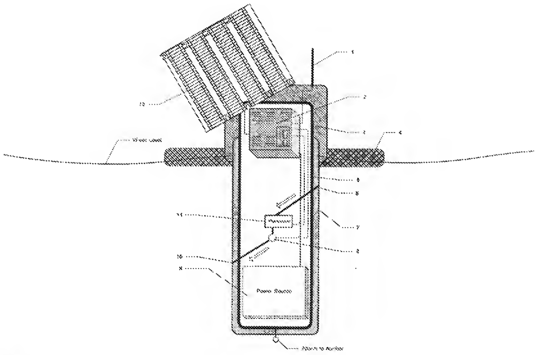


Figure 1

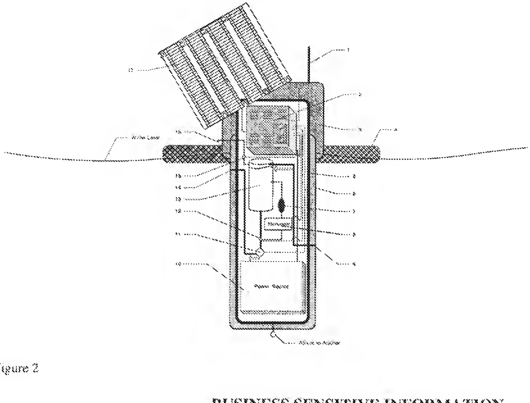


Figure 2

The AquaSentinel℠ Buoy system shows that biosensors based on fluorescence induction curves of naturally occurring freshwater algae can be used to detect cyanide, methyl parathion, and DCMU in primary water supplies under appropriate experimental conditions. In the context of current state-of-the-art biosensor research, they are unique: in the case of sunlight-exposed drinking water, the biosensors occur naturally in the medium to be protected. When combined with encrypted data telecommunication and a database-lookup library containing pertinent data for healthy algae, this approach to protection of sunlight-exposed primary drinking water supplies may be of practical value under real-world conditions.

Example 1. *Potassium Cyanide*

Hydrogen cyanide is a chemical warfare agent classified as a blood agent. It has an odor characteristic of bitter almonds and is completely miscible in water. For these experiments, the water-soluble salt potassium cyanide (KCN) was used. The cyanide ion is an extremely toxic and fast-acting poison. Food and drinking water are the main sources of cyanide exposure for individuals not subjected to occupational exposures (Guidelines for Canadian Drinking Water Quality, 1996). Typical symptoms of cyanide poisoning are headache, nausea, weakness, palpitations, tremors, and breathlessness. In cases of severe poisoning, the nervous and respiratory systems are the first to fail. With high levels of exposure, death results from respiratory arrest. The U.S. Army has proposed field drinking water standards for cyanide of 2 and 6 mg/L, assuming a water consumption of 15 and 5/L day, respectively (Guidelines for Chemical Warfare Agents in Military Field Drinking Water, 1995).

The effect of 2 mM KCN was tested on the fluorescence emission of "as is" water samples containing naturally-occurring algae from the Clinch River. The Clinch River is the main source of drinking water for Oak Ridge, Tennessee. After an initial control (no KCN) fluorescence measurement, KCN was added directly into the water sample. Figure 3 shows the change in the fluorescence induction curve after 2, 10 and 15 min exposure of the algae to KCN (red, green and blue lines respectively), compared to the control (black line). The photosynthetic biosensors can detect cyanide concentrations well below the minimum level for human toxicity. Based on consumption of 100 ml, the AquaSentinel℠ Buoy fluorometric sensing technique can detect levels of cyanide that are more than six times less than the minimum lethal dose reported by Gettler and Baine (1938) and nearly 20 times less than the $LD_{50}$ value.

Example 2. *Methyl Parathion*

Methyl Parathion (MPt) is an organophosphorus insecticide used to control soil-dwelling pests and a wide range of insects and mites that infest agricultural crops. It is a cholinesterase inhibitor that is structurally and functionally similar to the chemical warfare agents classified as nerve agents (including VX and GA). Severe exposure in humans and animals can lead to convulsions, unconsciousness, cardiac arrest, and death (Guidelines for Canadian Drinking Water Quality, 1996).

The effect of 20 $\mu$M MPt was tested on the fluorescence emission of "as is" water samples containing naturally-occurring algae from the Clinch River in Oak Ridge, Tennessee. After an initial control (no MPt) fluorescence measurement, MPt was added directly into the water sample. Figure 4 shows the change in the fluorescence induction curve after 2, 10 and 15 min exposure of the algae to MPt (red, green and blue lines respectively), compared to the control (black line). The photosynthetic biosensors can detect methyl parathion concentrations well below the minimum level for human toxicity. The MPt minimum detected level by the AquaSentinel℠ Buoy fluorometric sensing technique is 0.005 ppm when compared to a 0.3 ppm one-day and ten-day exposure for a 10-Kg child as established by the Environmental Protection Agency (Drinking Water Standards and Health Advisories, 2002).

Example 3. *DCMU (N'-(3,4-dichlorophenyl)-N,N-dimethylurea, Diuron)*

DCMU (Diuron) is a substituted urea-based herbicide employed principally for control of vegetation in noncrop areas, including irrigation and drainage ditches. Diuron is a nonionic compound with moderate water solubility. The U.S. Environmental Protection Agency has ranked Diuron fairly high (i.e., as a Priority B Chemical) with respect to potential for groundwater contamination. Diuron is of low acute toxicity (Guidelines for Canadian Drinking Water Quality, 1996).

The effect of 10 $\mu$M DCMU was tested on the fluorescence emission of "as is" water samples containing naturally-occurring algae from the Clinch River in Oak Ridge, Tennessee. After an initial control (no DCMU) fluorescence measurement, DCMU was added directly into the water sample. Figure 5 shows the change in the fluorescence induction curve after 2, 10 and 15 min exposure of the algae to DCMU (red, green and blue lines respectively), compared to the control (black line). The photosynthetic biosensors can detect methyl parathion concentrations well below the minimum level for human toxicity. The DCMU minimum detected level by the AquaSentinel℠ Buoy fluorometric sensing technique is 0.002 ppm when compared to a 1 ppm one-day and ten-day exposure for a 10-Kg child as established by the Environmental Protection Agency (Drinking Water Standards and Health Advisories, 2002).

A summary of the decrease in photochemical yields measured at 0°C with "as is" water samples containing naturally-occurring algae from the Clinch River in Oak Ridge, Tennessee is illustrated in Table 1. These results show the AquaSentinel[SM] Buoy fluorometric sensing technique is effective in detecting the presence of these toxic agents in primary-source drinking water at such a low temperature.

Table 1. Percentage (%) decrease in photochemical yield at 0°C for naturally-occurring algae from water samples of the Clinch River, Oak Ridge, Tennessee.

| Time after exposure (mins) | 2 mM Potassium Cyanide (KCN) | 20 $\mu$M Methyl Parathion (MPt) | 10 $\mu$M DCMU |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 2 | -0.11 | -3.19 | -8.87 |
| 10 | -10.95 | -5.41 | -19.43 |
| 15 | -20.08 | -7.80 | -22.83 |

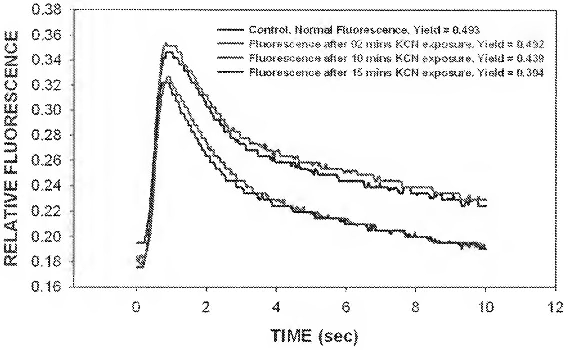


Figure 3

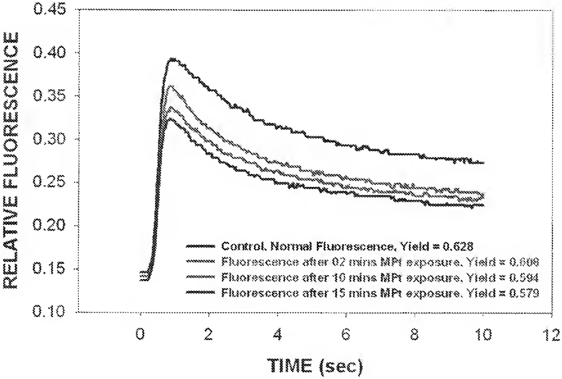


Figure 4

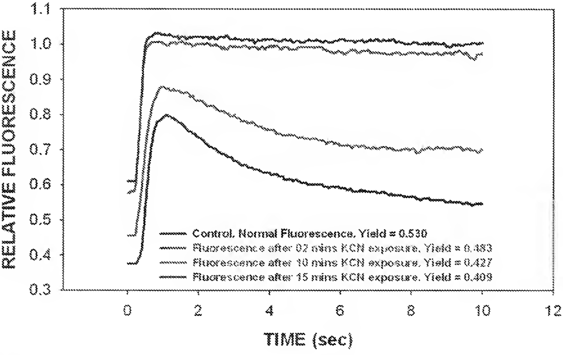


Figure 5

6. RELATED TECHNOLOGY:


Baxter, Jr., J.F., 1996. Oil spill detection system. U.S. Patent #5,532,679.

Baxter, Jr., J.F., Bailey, D.F., 1997. Tracking buoy. U.S. Patent #5,654,692.

Drinking Water Standards and Health Advisories, 2002. EPA 822-R-02-038. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

Dubelaar, G.B.J., Gerritzen, P.L., Beeker, A.E.R., Jonker, R.R., Tangen, K., 1999. Design and first results of CytoBuoy: a wireless flow cytometer for an in situ analysis of marine and fresh waters. Cytometry 37: 247-254.

Grana, D.C., Haynes, D.P., 1978. Remote water monitoring system. U.S. Patent #4,089,209.

Gelda, R.K., Effler, S.W., 2002. Estimating oxygen exchange across the air-water interface of a hypereutrophic lake. Hydrobiologia 487: 243-254.

Gettler, A., Baine, J., 1938. The toxicology of cyanide. Am. J. Med. Sci. 195: 182-198.

Greenbaum, E., Sanders, C., Tissue-based standoff biosensors of detecting chemical warfare agents. U.S. Patent Application, Docket 0815.2

Greenbaum, E., Sanders, C., Tissue-based water quality biosensors of detecting chemical warfare agents. U.S. Patent Application. Publication Number US 2002/0102629.

Guidelines for Canadian Drinking Water Quality, 6th ed., 1996. Ministry of Supply and Services, Ottawa, Canada.

Guidelines for Chemical Warfare Agents in Military Field Drinking Water, 1995. National Research Council, National Academy Press, Washington, D.C.

Kishino, M., Ishizaka, J., Saitoh, S., Senga, Y., Utashima, M., 1997. Verification plan of ocean color and temperature scanner atmospheric correction and phytoplankton pigment by moored optical buoy system. J. Geophys. Res., D. 102: 17197-17207.

Klett, J.W., Edie, D.D., 1995. Flexible towpreg for the fabrication of high thermal conductivity carbon/carbon composites. Carbon 33: 1485-1503.

Klett, J., Hardy, R., Romine, E., Walls, C., Burchell, T., 2000. High-thermal-conductivity, mesophase-pitch-derived carbon foams: effect of precursor on structure and properties. Carbon 38: 953-973.

Korde, U.A., 2000. A note on the hydrodynamics of a tail tube buoy. Ocean Eng. 27: 1473-1484.

Lapota, D., Andrews, J., Lieberman, S., Anderson, G., 2002. Development of an autonomous bioluminescence buoy (Biobuoy) for long-term ocean measurements. Proceedings of the Oceans '02 MTS/IEEE Conference 1: 396-401.

Lobsiger, U., Manoel, J.L., 2000. Installation for in situ monitoring the quality of habitat of aquatic organisms. U.S. Patent #6,119,630.

Moldaenke, C., 1993. Process and device for measuring algae fluorescence feedback. European Patents #WO09312415 and #EP0615618.

Moldaenke, C., Beutler, M., 2001. Measuring process for automatically determining the offset during fluorometric chlorophyll content determination and simultaneous determination of yellow material comprises directing an excited spectrum onto an aqueous phase. European Patent #DE19941415.

Moore, T., Zhang, K., Close, G., Moore, R., 2000. Real-time river level monitoring using GPS heighting. GPS Solut. 4: 63-67.

Ory, J.-M., 1997. Biosensor and method . controlling the water quality. European Patent 207811842.

Pinto, A.M.F., Von Sperling, E., Moreira, R.M., 2001. Chlorophyll-a determination via continuous measurement of plankton fluorescence: methodology development. Wat. Res. 35: 3977-3981.

Rodriguez, Jr., M., Sanders, C.A., Greenbaum, E., 2002. Biosensors for rapid monitoring of primary-source drinking water using naturally-occurring photosynthesis. Biosens. Bioelectron. 17: 843-849.

Sanders, C.A., Rodriguez, Jr., M., Greenbaum, E., 2001. Stand-off tissue-based biosensors for the detection of chemical warfare agents using photosynthetic fluorescence induction. Biosens. Bioelectron. 16: 439-446.

Sutherland, A.L., Swenson, R.C., 1984. Shallow water environmental/oceanographic measurement system. U.S. Patent #4,448,068.

Wild-Allen, K., Tett, P., Bowers, D., 1997. Observations of diffuse upwelling irradiance and chlorophyll in case I waters near the Canary Islands (Spain). Optics & Laser Technol. 29: 3-8

7. UNIQUE FEATURES:

The AquaSentinel[SM] Buoy represents a major and innovative addition to existing biosensor technology for water monitoring and protection. This new technology is designed to work with and markedly improve the effectiveness of high-end water analysis laboratory measurements.

- Unlike currently available bench-top biosensor technology, the ORNL technology provides a field-deployable instrument which is solar powered and thermally protected from temperature extremes.
- In addition, unlike the competition, the instrument is submersible and can be made mobile by incorporation into a minisubmarine vehicle
- Because there is an unlimited supply of biosensor material in the monitored environment, continuous around-the-clock monitoring of primary-source drinking water is possible with this revolutionary approach.
- An early warning alert of toxic agents is provided by the short turnaround time needed for analysis, that is, about 10 seconds to complete the fluorescence induction curve measurements. Thus, the biosensor technology can provide reports to data analysis centers in real time via wireless encrypted telecommunications, providing an early warning alert that reports the location and time of a suspected chemical attack. Figure 7 illustrates this process.

8. POSSIBLE ALTERNATIVE VERSIONS:

The AquaSentinel[SM] Buoy could include versions powered by a hydrogen fuel cell, wave motion or other electrical power technologies that would improve efficiency.

9. PROBABLE USES:

Probable uses include chemical or biological terrorism detection; industrial or residential waste contamination; agricultural pesticides detection and drinking water/reservoir monitoring.

10. REDUCTION TO PRACTICE:

☐ Invention is purely conceptual and needs experimentation to validate the concept.

☐ Invention is conceptual, but does not need experimentation to validate the concept.

☒ Proof-of-principle experiment has been performed to validate the concept.

☒ Invention has been demonstrated on a [ ☒ laboratory; ☒ prototype; ☐ production ] scale.

☐ Other (Explain):

## PART II - FACTS RELATING TO THE INVENTION

11. SOURCE(S) OF FUNDS:

☒ DOE B&R Code: 600301010

☐ 100% funds-in from third party identified below

☐ LDRD

☐ Seed Money

☒ Other (explain): United Defense?, DARPA?

Identify respective Program Manager: Richard L. Stouder?, Alan Rudolph ?

12. THIRD PARTY RIGHTS IN THE INVENTION:

Is a third party employer or sponsor involved in the invention? ☐ YES ☒ NO

If yes, provide the following information and provide copies of documents.

☐ CRADA No.

☐ Subcontract No.

☐ Interagency Agreement No.

☐ Work For Others No.

☐ Non-employee -- Inventor is acting as agent or representative (explain):

☐ No employer-employment relationship or written agreement (explain):

☐ Other (explain):

Name and address of third party:

Effective dates of contract or agreement:

Explain any special circumstances:

13. SUBMITTERS:

**A.** Full Name David E. Hill [redacted] Citizenship: USA

Residence Address: 8224 Broken Arrow, Knoxville TN 37923 Telephone: 865-691-2428

Current Employer: ☒UT-Battelle ☐Other →

Employee No.: 33366 Work Address: 4500S-B159, MS-6140 Telephone: : 865-576-9899

UT-Battelle Division No.: 011 Division Name: Metals and Ceramics Division Manager: E. E. Bloom Supervisor: R. B. Ogle

My specific contribution to the concept of the invention is: Originated AquaSentinelSM Buoy concept. Developed the layout of the buoy's functional components and contributed to the design by tying different technologies together to make the buoy work in all enviromental conditions.

I recorded my contribution in Notebook # Page(s) Date Witnessed by.

My employer at that time was: ☒Same as above ☐Other→

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document:

Signature: ______________________ Date: ______________

---

**B.** Full Name Miguel Rodriguez, Jr [redacted] Citizenship: USA

Residence Address: 200 Elmhurst Drive, Apt. I-37 Oak Ridge TN 37830 Telephone: 865-482-1139

Current Employer: ☒UT-Battelle ☐Other →

Employee No.: 35468 Work Address: 4505-23B, MS-6226 Telephone: : 865-241-4957

UT-Battelle Division No.: 1 Division Name: Life Sciences Division Manager: Barry A. Berven Supervisor: Brian H. Davison

My specific contribution to the concept of the invention is: Instructed Dave Hill on AquaSentinel's prototype system . Provided information used for the specifications and organization of functional components based from direct involvement in the design and complete set-up of first working AquaSentinelSM field prototype.

I recorded my contribution in Notebook # Page(s) Date Witnessed by:

My employer at that time was: ☒Same as above ☐Other→

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document:

Signature: ______________________ Date: ______________

C. Full Name Elias Greenbaum [redacted] Citizenship: USA

Residence Address: 11403 Morgan Overlook Dr., Knoxville, TN 37931 Telephone: 865-927-4488

Current Employer: ☒UT-Battelle ☐Other →

Employee No.: 21303 Work Address: 4500N, A4, MS-6194 Telephone: : 865-574-6835

UT-Battelle Division No.: 60 Division Name: Chemical Sciences Division Manager: Michelle Buchanan Supervisor: Michelle Buchanan

My specific contribution to the concept of the invention is. Invented AquaSentinelSM concept for primary source water protection. Instructed Dave Hill on how AquaSentinel works. Answered questions about the integration of the buoy into the AquaSentinel system. I also assisted Miguel in set-up of field prototype.

I recorded my contribution in Notebook # Page(s) Date Witnessed by:

My employer at that time was: ☒Same as above ☐Other →

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document:

Signature: ______________________________ Date: ________________

---

D. Full Name James W. Klett. [redacted] Citizenship: USA

Residence Address: 10073 Delle Meade Dr. Knoxville TN 37931 Telephone: 865-691-9742

Current Employer: ☒UT-Battelle ☐Other →

Employee No.: 35624 Work Address: 4508-144, MS-6087 Telephone: : 865-574-5220

UT-Battelle Division No.: 11 Division Name: Metals and Ceramics Division Manager: E. E. Bloom Supervisor: T. D. Burchell

My specific contribution to the concept of the invention is: Provided information on thermal conductivity implementation on the buoy housing based on ORNL's graphite fiber composite propietary technology.

I recorded my contribution in Notebook # Page(s) Date Witnessed by:

My employer at that time was: ☒Same as above ☐Other →

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document:

Signature: ______________________________ Date: ________________

---

If there are additional submitters, provide above information on a separate paper.

14. NOTEBOOK RECORDS:

| EVENT | DATE | NOTEBOOK NO. | PAGES | TWO NOTEBOOK WITNESSES Inventors cannot be witnesses | WITNESS DATES |
|---|---|---|---|---|---|
| Initial Concept | | | | 1. Everett Bloom | |
| | | | | 2. Richard Stouder | |
| First Sketch or Drawing | | | | 1. Everett Bloom | |
| | | | | 2. Richard Stouder | |
| First Written Description | | | | 1. | |
| | | | | 2. | |
| First Model or Test Unit | | | | 1. | |
| | | | | 2. | |
| First Test of Invention | | | | 1. | |
| | | | | 2. | |

List any other permanent records of the invention:

15. PUBLICATION STATUS:

Has the invention been disclosed to the public or any party outside DOE and UT-Battelle?

☒YES ☐NO NOT A PUBLIC DIVULGATION JM

If yes, indicate the form(s) of the disclosure: ☒Oral ☒Visuals ☐Abstract ☐Full Article ☐Other

☐Submitted for review, but not yet published

Date of Conference or Publication: Location of Conference: Journal:

Other relevant information Only to United Defense that sponsored the project

16. ROUTINE USE OF THE INVENTION:

If the inventors have tested any embodiment of the invention, has there been any additional, routine use of the invention?

☐YES ☒NO

If yes indicate date and circumstances of first such use:

17. SALE OF THE INVENTION:

Has there been any sale of the invention or any offer to sell the invention?

☐YES ☒NO

If yes indicate date and circumstances:

**STOP here. Print this document, obtain classification check and submitters' signatures, and forward to OGC/IP.**

18. CLASSIFICATION CHECK:

This document is properly classified as:

____Confidential ____Secret ____Restricted Data ____Formerly Restricted Data ____National Security Information

____Unclassified (Contains no classified information) ____DUSA (Designated Unclassified Subject Area)

Signature (required):__________ Date: __________

19. RECEIPT BY OFFICE OF GENERAL COUNSEL, INTELLECTUAL PROPERTY:

Received by: __________ Date: MAY 16 2003